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Symposium on ‘Nutrition in early life: new horizons in a new century’

Session 4: Mineral metabolism and body composition Iron status of breast-fed infants

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Fe deficiency is a common nutritional disorder during infancy, particularly in low-income countries. The Fe status of a breast-fed infant is strongly influenced by the body Fe content at birth, which is determined by factors that operate before birth (maternal Fe status before and during pregnancy; infant gestational age and birth weight) and at the time of delivery (the timing of umbilical cord clamping). Delaying the clamping of the umbilical cord for 2 min can increase body Fe content by approximately 33% (75 mg), and results in greater Fe stores at 6 months of age. After birth, male gender and a rapid rate of weight gain are associated with lower Fe status. During the first half of infancy dietary Fe requirements depend on Fe stores at birth. For an exclusively-breast-fed full-term normal-birth-weight infant with delayed umbilical cord clamping, whose mother had adequate Fe status during pregnancy, the Fe provided from stores and breast milk is sufficient for ≥ 6 months, but before this time higher-risk infants may become Fe deficient. Fe supplementation can be beneficial for high-risk infants, but can have adverse effects on growth and morbidity of Fe-replete infants. After 6 months most breast-fed infants will require complementary foods that are rich in Fe.

Iron status: Breast-fed infants: Timing of umbilical cord clamping: Iron requirements in infancy

Fe deficiency and Fe-deficiency anaemia (IDA) are common among infants and young children. Estimates of the incidence of IDA in children by 1 year of age are as high as 50% in developing countries (Gillespie & Johnston, 1998). IDA is of particular concern in infants because of its potentially detrimental effects on mental, motor and behavioural development, some of which may be irreversible even after treatment to correct the deficiency. As breast milk contains little Fe (even though its bioavailability is high), Fe deficiency can occur in breast-fed infants.

The present paper will cover several issues related to the Fe status of breast-fed infants. First, the factors affecting infant Fe status will be reviewed, with an emphasis on the role of the timing of umbilical cord clamping, which is a factor that until very recently had received little attention.

Second, Fe requirements during infancy will be examined, using a new approach for calculating these requirements that takes into account the infant's birth weight and the timing of clamping of the umbilical cord. Third, the results of a two-country Fe-supplementation trial of breast-fed infants will be summarized, including: (a) effects on Fe status, anaemia, growth and morbidity; (b) gender differences in Fe status; (c) recommendations regarding the diagnostic criteria for Fe deficiency and anaemia.

Factors affecting infant iron status

Fetal iron accretion and erythropoiesis

During gestation fetal erythropoietic activity is high. The fetus requires a large erythrocyte mass to provide

Abbreviations: BW, body weight; IDA, Fe-deficiency anaemia; TBI, total body Fe; ZPP, zinc protoporphyrin.
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sufficient O₂ for development and to overcome the relatively lower O₂ availability found *in utero*. Thus, at birth Hb concentration and packed cell volume are higher than at any other time in life, reflecting the gradual increase in erythrocyte production during gestation. Hb concentration is approximately 90 g/l at 9–10 weeks of gestation, increasing to 140 g/l by mid-gestation and 170 g/l (the average value at birth) by the last 6–8 weeks of gestation (Brown, 1988). The primary Hb found in fetal erythrocytes is different from adult Hb, both in its globin chains and physiological properties. Fetal Hb has a higher affinity for O₂, as a result of a lower affinity to 2,3-diphosphoglycerate, which stabilizes the deoxygenated state (Bard, 2000). The increased O₂ affinity of fetal Hb is important because of the relatively hypoxic conditions *in utero* and the importance of adequate oxygenation for the growing fetus. As the fetus nears term fetal Hb production decreases and adult Hb production increases, reaching approximately 98% total Hb by 20–30 weeks postnatally (Bard, 2000).

The details of placental Fe transport are poorly understood. Transferrin-bound Fe is taken up by the transferrin receptor expressed on the syncytiotrophoblast membrane of the placenta (Douglas & King, 1990), taken into the cell and released into the cytoplasm in a process most likely involving divalent metal transporter 1 (Georgieff *et al.* 2000). It is thought that ferroportin is involved in the pathway that takes the released Fe into the fetal circulation (Gambling *et al.* 2001). The placenta removes Fe from the maternal circulation for the fetus at the expense of maternal Fe stores, and in placental cells cultured with low Fe levels and in pregnant rats fed Fe-deficient diets the expression of Fe transport proteins is increased (Gambling *et al.* 2001). Despite this compensation in placental Fe transport to the fetus in the presence of maternal Fe deficiency, the infant's Fe accretion still appears to suffer. It was long believed that only severe IDA during pregnancy affects fetal Fe stores; in fact, some studies (Siimes & Siimes, 1986; Harthoorn-Lasthuizen *et al.* 2001) have shown no differences in neonatal Fe status indices between infants born to Fe-deficient and Fe-replete mothers. However, recent studies with longer-term follow-up (Colomer *et al.* 1990; Kilbride *et al.* 1999; de Pee *et al.* 2002) have shown that infants born to Fe-deficient anaemic mothers have a higher incidence of Fe deficiency and anaemia later in infancy than infants born to Fe-replete mothers.

The infant's gestational age and birth weight strongly affect the size of the infant's Fe stores at birth. Although the concentration of Fe in the liver, spleen and kidney remains relatively constant during gestation, there is an increase in total Fe content as a result of growth of the Fe storage organs (Singla *et al.* 1985). The last 8 weeks of gestation are particularly important for the increase in total Fe in the liver. The increase in fetal Fe stores with gestational age is reflected in increasing serum ferritin concentrations in fetal cord blood as gestation nears term (Siimes & Siimes, 1986).

Timing of umbilical cord clamping

The timing of clamping of the umbilical cord can have a profound effect on blood volume and hence the total body

Fe (TBI) content of the newborn. *In utero* the total fetal-placenta circulation is approximately 110–115 ml/kg fetal body weight (BW) with approximately 70 ml/kg BW in the fetus and 45 ml/kg BW in the placenta (Linderkamp, 1982). The blood volume of the infant immediately after birth will depend largely on the amount of placental transfusion that is allowed to occur. The amount of blood transferred from the placenta to the infant is dependent on the rate of transfer and the time at which the cord is clamped. Other factors that come into play are gravity and uterine contractions. For a period of time after birth there is still circulation between the infant and placenta through the umbilical vein and arteries. Closure of the umbilical vessels depends on various factors, including levels of hormones and PG, temperature and O₂ saturation of cord blood. By measuring placental residual blood volume after clamping the umbilical vein and/or arteries at various time points, it has been shown that blood flows through the umbilical arteries (from the infant to the placenta) during the first 20–25 s after birth but is negligible by about 40–45 s (Yao & Lind, 1974). In contrast, in the umbilical vein blood flow continues from the placenta to the infant up to 3 min after delivery, after which blood flow is insignificant. Pulsations in the cord, which are frequently used as a judge of when to clamp, are pulsations from the infant's heart that can be felt in the umbilical artery and occur for several min (generally 1–5) after birth.

The rate of placental transfusion is rapid at first and then slows in a stepwise fashion, with approximately 25% of the transfer occurring in the first 15–30 s after the uterine contraction of birth, 50–78% of the transfer by 60 s and the remaining transfer by 3 min (Yao *et al.* 1969). Not all placental blood is transferred to the infant; a small percentage of the total fetoplacental circulation remains in the placenta, probably preventing blood volume overload of the infant (Linderkamp, 1982). Uterine contraction, which naturally occurs again between 1 and 3 min after the birth contraction, is thought to be responsible for the last 'step' of the placental transfer (Yao *et al.* 1968). Uterine contraction can accelerate the rate of transfer; when methylergonovine (an oxytocic drug) is given immediately after birth, placental blood transfer occurs in 1 min, after a uterine contraction occurs at approximately 45 s (Yao *et al.* 1968). Gravity can also play a role; if the infant is held sufficiently high enough above the mother's uterus (500–600 mm in one study) placental transfusion can be prevented by stopping blood flow through the umbilical vein, and possibly allowing backflow through the umbilical arteries (Yao & Lind, 1974). When the infant is placed on the mother's abdomen until cord pulsations cease, the amount of blood transferred to the infant is thought to be intermediary; more than with immediate clamping but not as much as when the infant is held at the level of the uterus (Nelle *et al.* 1995). If the infant is held below the level of the uterus gravity seems to speed the rate of transfer, but does not seem to change the total amount of blood transferred.

Several attempts have been made to measure the changes in infant blood volume after different clamping times. Six studies carried out from the 1940s to the 1960s used radioisotope-dilution techniques to estimate infant

blood volume (DeMarsh *et al.* 1942; Whipple *et al.* 1957; Usher *et al.* 1963; Oh *et al.* 1966; Yao *et al.* 1969; Saigal *et al.* 1972). There is inconsistency in the definitions of late clamping used; two studies clamped at 3 min, two at the end of cord pulsations (which occurred at approximately 5 min in one study), one at 5 min and one at placental descent into the vagina. However, it can be assumed that the placental transfer is essentially complete at 3 min, which will probably encompass the late-clamped infants in all studies. The estimated average placental blood transfer with delayed clamping was shown to be 18 (range 11–26) ml/kg BW or a 13–32% increase in blood volume as compared with immediate clamping. However, results obtained using this method can be affected by the time at which blood volume measurements are completed. As blood volume is tightly controlled, the increase in infant blood volume after receiving a placental transfusion is rapidly adjusted in the first hours after birth by a plasma shift from the intravascular to the extravascular spaces (and a consequent rise in packed cell volume; Oh *et al.* 1966). Thus, studies that measure blood volume close to birth (within the first hour) give higher and possibly more accurate estimates of the total placental transfusion. In two of the six studies blood volume was measured as close as possible to delivery, one at 7 min (Oh *et al.* 1966) and one at 0.5 h (Usher *et al.* 1963), while in the others (DeMarsh *et al.* 1942; Whipple *et al.* 1957; Yao *et al.* 1969; Saigal *et al.* 1972) the first measurements of blood volume were made after 4 h. The difference between clamping groups in the two former studies is similar: 26 and 21 ml blood/kg BW. Both groups of authors used their data to back-extrapolate to within minutes of birth and have estimated that the actual transfer was 42–48 ml blood/kg BW. In a more recent study (Linderkamp *et al.* 1992), using a different technique, the investigators measured the amount of infant Hb in the homogenized placenta and in cord blood to estimate residual placental blood volume, and then subtracted this value from an assumed fetal–placental blood volume of 115 ml/kg BW to estimate infant blood volume at birth. In this study the amount of blood transferred after a 3 min delay in cord clamping was found to be 35 ml/kg BW, which represents a 50% increase in total blood volume as compared with infants who underwent immediate clamping. This study and the two earlier studies (Usher *et al.* 1963; Oh *et al.* 1966) that utilized the radioisotope-dilution technique as soon as possible after birth indicate that the placental transfusion is 35–48 ml/kg BW. The approximate midpoint of this range, 40 ml/kg BW, represents an increase of about 50% in the total blood volume of the newborn. Placing the infant on the mother's abdomen before delayed clamping reduces this transfer to approximately 20 ml/kg BW (Nelle *et al.* 1995).

Assuming a Hb concentration of 170 g/l at birth, and 3.47 mg Fe/g Hb, a placental transfusion of 40 ml/kg BW would transfer 128 ml blood and 75 mg Fe for a 3.2 kg infant. Theoretically, such a substantial endowment of Fe should have a measurable effect on the long-term status of the infant, which may or may not be detectable through haematological indicators alone. Seven studies have examined the effect of the timing of cord clamping on

these outcomes in full-term infants (Wilson *et al.* 1941; Lanzkowsky, 1960; Pao-Chen & Tsu-Shan, 1960; Geethanath *et al.* 1997; Grajeda *et al.* 1997; Gupta & Ramji, 2002; Chaparro *et al.* 2006); the four most recent studies examined both Fe and haematological status, while three studies conducted several decades ago measured indicators of haematological status only. In Guatemalan infants (Grajeda *et al.* 1997) delayed clamping (defined as waiting until pulsations in the cord ceased) was found to increase Hb concentration at 2 months of age by 7.7 g/l. The percentage of infants with a packed cell volume of <33% was reported to be 42 in the delayed-clamping group v. 88 in the early-clamping group. No significant difference in Fe status (ferritin, serum Fe, total Fe-binding capacity or transferrin saturation) was found, although ferritin tended to be higher in the delayed-clamping group. Two studies in India (Geethanath *et al.* 1997; Gupta & Ramji, 2002), both of which measured infant Fe status at 3 months of age, have shown conflicting results. One study (Gupta & Ramji, 2002) has shown a beneficial effect of delayed clamping in infants born to anaemic mothers (Hb < 100 g/l) on both infant Hb and serum ferritin. In this study the odds of developing anaemia (<100 g/l) by 3 months of age was reported to be 7.7 times higher in the early-clamped group as compared with the late-clamped group. The other study (Geethanath *et al.* 1997), of infants born to mothers with Hb > 100 g/l, has shown no significant effect of clamping time on infant Hb or serum ferritin, although a non-significant trend towards higher ferritin was found in the delayed-clamped group. A study from South Africa published in 1960 (Lanzkowsky, 1960) has shown no significant difference in Hb between clamping groups; however, no other indicators of Fe status were measured. Only three studies (Wilson *et al.* 1941; Pao-Chen & Tsu-Shan, 1960; Chaparro *et al.* 2006) have assessed the effect of cord clamping time on infant status after 3 months of age. In a study from China (Pao-Chen & Tsu-Shan, 1960) no significant differences were found in Hb concentration at 6 months of age. Similarly, a US study (Wilson *et al.* 1941), in which twenty-eight infants were studied to 9 months of age, failed to demonstrate a difference in Hb concentration between early- and delayed-clamped groups, although mean corpuscular Hb was found to be higher in the delayed-clamped group (with delayed clamping defined as at placental separation from the uterus). In both these latter studies, however, no other indicators of Fe status were measured and there was no control for potentially-confounding factors such as feeding practices and growth. The most recent study is a randomized controlled trial of 476 infants born in Mexico City and followed to 6 months of age (Chaparro *et al.* 2006). Infants who underwent delayed umbilical cord clamping (at 2 min after delivery) were found to have a higher mean corpuscular volume, ferritin concentration and TBI at 6 months than infants whose umbilical cords were clamped immediately (approximately 17 s after delivery). The difference found in body storage Fe between clamping groups is equivalent to >1 month of Fe requirements. The difference was found to be even greater in infants who had a birth weight of <3000 g, were born to mothers with Fe deficiency or did not receive Fe-fortified formulas or milks. From an exit

survey completed with obstetrics residents participating in the randomized controlled trial (n 30), 87% were willing to practice delayed clamping if the practice proved beneficial for the infant and, at press, hospital physicians were considering adopting a policy of delayed cord clamping.

Postnatal factors affecting iron status

The first half of infancy is a period of dynamic haematological changes, as the infant adapts to the extrauterine environment. As a result of the newborn's high Hb concentration and the relatively higher O₂ availability outside the womb, erythropoietin production and erythropoiesis decline after birth. Hb concentration decreases from approximately 170 g/l at birth to a low of 112 g/l (Brown, 1988) at about 2 months of age. This 'physiological anaemia of infancy' is a result of a combined effect of the shorter lifespan of fetal erythrocytes, decreased erythrocyte production and a dilution effect from increased blood volume related to growth. From 2 months onward Hb usually begins increasing again, reaching an average value of approximately 118 g/l in breast-fed infants (Domellöf *et al.* 2001; a 'typical' Hb concentration for infants receiving Fe-fortified formula or supplements would be higher).

These physiological changes are accompanied by a redistribution of body Fe. Newborns are thought to have approximately 75 mg Fe/kg BW (approximately 260 mg total; Widdowson & Spray, 1951) of which approximately 70% is in Hb, 24% is in stores as ferritin and the remaining 6% is in myoglobin and Fe-containing enzymes (Dallman *et al.* 1993). By 4 months of age there is little change in TBI content, but as Fe stores are mobilized for growth and serum ferritin concentrations decline, the percentage shifts to 76 in Hb, 12 in stores and 12 in myoglobin and enzymes (Dallman *et al.* 1993). After 4–6 months of age, when the breast-fed infant begins receiving an exogenous source of Fe, TBI content increases to approximately 420 mg by 12 months of age, with the same relative percentages in Hb, stores and myoglobin and enzymes.

The rate at which Fe in stores is depleted during the first half of infancy depends on the demand for Fe to support growth (a function of increased blood volume and gain in lean body mass) and Fe intake from human milk and other sources. Accordingly, infants with a rapid rate of weight gain are at higher risk for Fe deficiency (Michaelsen *et al.* 1995; Thorsdottir *et al.* 2003). Fe intake from breast milk is minimal, as human milk has an Fe concentration of only approximately 0.35 mg Fe/l (Institute of Medicine, 1991). An infant consuming 750–800 ml breast milk/d would receive approximately 0.27 mg Fe/d. There have been several attempts to measure the percentage absorption of Fe from human milk. Older studies using radioisotope tracers (Gorten *et al.* 1963; Saarinen *et al.* 1977) have suggested that 32–49% breast-milk Fe is absorbed, whereas studies in the 1990s using stable-isotope tracers (Davidsson *et al.* 1994; Abrams *et al.* 1997) have yielded lower estimates of 12–21%. Subsequent stable-isotope studies have indicated that Fe absorption from human milk varies with age and Fe status. In Swedish infants who had

not received any Fe supplements (Domellöf *et al.* 2002b) the mean absorption was found to be 15.7% at 6 months and 36.7% at 9 months, with values of 11.9% and 16.9% respectively for infants who had received Fe supplements. Among infants in Peru (Hicks *et al.* 2006), who are likely to be more Fe deficient than the infants in Sweden, Fe absorption from human milk was reported to be 42.6% at 5–6 months (while exclusively breast-fed) and 51.9% at 9–10 months. The rates of absorption in Peru were related to the infant's Fe status; 56.4% in those with a serum ferritin concentration of <12 µg/l v. 38.0% in those with a serum ferritin of >12 µg/l (age-groups combined). Thus, the variability in previous estimates of Fe absorption from human milk could be a result not only of methodological factors, but also of differences in infant age and Fe status across studies.

Given a range of 12–56% in Fe absorption from human milk, exclusively breast-fed infants consuming an average of 0.27 mg Fe/d would absorb 0.03–0.15 mg Fe/d. There is some evidence that the introduction of complementary foods or liquids that are low in Fe will decrease the bioavailability of Fe from human milk (Saarinen & Siimes, 1979; Oski & Landaw, 1980). Thus, exclusive breast-feeding during the first 6 months may be protective against Fe deficiency in populations in which Fe-fortified formulas or food are not commonly used. In developing countries complementary foods and liquids may also introduce pathogens that can potentially increase Fe losses through increased morbidity or, most importantly, increased diarrhoeal episodes, which cause increased epithelial cell sloughing and/or bleeding. A common early complementary food introduced in many countries, fresh cow's milk, may increase Fe loss in early infancy by causing gastrointestinal bleeding (Fomon *et al.* 1981). On the other hand, Fe intake can be many-fold greater among infants who receive Fe-fortified formulas or foods, and for this reason some studies (Meinzen-Derr *et al.* 2006) have shown a higher risk of Fe deficiency among exclusively breast-fed infants than in those fed both breast milk and infant formula.

After 6 months of age, when complementary foods should be part of the infant's diet, the bioavailable Fe content of those foods is a key determinant of Fe status. At this age Fe stores are likely to be depleted in a substantial proportion of breast-fed infants, and because the amount of Fe in breast milk is low 98% of the Fe needs must come from other sources (Dewey, 2005).

Iron requirements during infancy

There are two approaches to calculating Fe requirements during infancy. One is a simple factorial model, by which the amount of absorbed Fe required is the sum of estimated daily Fe losses plus the Fe required for growth. This approach assumes no net utilization of Fe from stores, i.e. the amount remaining in stores would stay the same as at birth. However, this assumption is not valid during early life because newborns are generally born with abundant Fe in stores that can be used to meet Fe needs during the first several months. An alternative approach is to

Table 1. Infant iron requirements from birth to 6 months, by birth weight and clamping time

Birth weight (kg)	3.2		3.5	
	Early	Delayed	Early	Delayed
Birth				
Fe _{circ0} (mg)	141.6	141.6	154.8	154.8
Fe _{myo/en0} (mg)	28.3	28.3	31.0	31.0
Fe _{stores0} (mg)	56.6	56.6	61.9	61.9
Fe _{PT} (mg)	7.0	75.0	8.0	83.0
TBI at birth (mg)	233.5	301.5	255.8	330.8
6 months				
Weight* (kg)	7.6		7.6	
Fe _{circ6} (mg)	233.4	233.4	233.4	233.4
Fe _{myo/en6} (mg)	46.7	46.7	46.7	46.7
Fe _{stores6} (mg)	38.0	38.0	38.0	38.0
TBI at 6 months (mg)	318.1	318.1	318.1	318.1
Fe losses, 0–6 months (mg)	33	33	33	33
Absorbed Fe requirements†, 0–6 months				
TBI at birth (mg)	233.5	301.5	255.8	330.8
Fe needed to maintain TBI at 6 months‡ (mg)	351.1	351.1	351.1	351.1
Change in body Fe, 0–6 months (mg)	117.5	49.5	95.3	20.3
Absorbed Fe requirement (mg/d)	0.64	0.27	0.52	0.11
Net Fe requirements§, 0–6 months				
Breast-milk Fe absorbed over 6 months (mg): 12%	5.5	5.5	5.5	5.5
56%	27.4	27.4	27.4	27.4
Net Fe requirement over 6 months (mg): 12%	112	44.0	89.8	14.8
56%	90.1	22.1	67.9	0
Net Fe requirement (mg/d): 12%	0.61	0.24	0.49	0.08
56%	0.49	0.12	0.37	0

Fe_{circ0}, Fe_{circ6} Fe in circulation as Hb at 0 and 6 months respectively; Fe_{myo/en0}, Fe_{myo/en6}, Fe needed for myoglobin and other Fe-containing enzymes at 0 and 6 months respectively; Fe_{stores0}, Fe_{stores6}, amount of storage Fe that is thought to be adequate at 0 and 6 months respectively; Fe_{PT} Fe contributed to TBI from placental transfusion; TBI, total body Fe.

*Weight at 6 months (7.6 kg) is the average of the median weight-for-age for girls and boys from the WHO Child Growth Standards (World Health Organization Multicentre Growth Reference Study Group, 2006).

†Based on change in TBI, and assuming Fe stores of approximately 10% TBI.

‡Accounting for 6 months of Fe losses (0.18 mg/d).

§From non-breast-milk sources; accounting for absorbed breast milk Fe, based on a range of bioavailability (12% v. 56%).

calculate the amount of absorbed Fe required on the basis of the expected change in TBI from birth, assuming normal levels of Hb, muscle growth and a certain percentage of Fe remaining in stores, and adding in an allowance for daily Fe losses (Fomon, 1995). In both approaches the dietary Fe requirement is calculated by dividing the amount of absorbed Fe needed by the estimated proportion absorbed.

The second (TBI) approach depends greatly on the estimated value of Fe stores at birth. As explained previously, this value is strongly influenced by birth weight and the timing of clamping of the umbilical cord (and also by maternal prenatal Fe status, although quantifying the influence of this factor is more difficult). The Fe requirements for the first 6 months of life will be presented using the TBI approach for four different scenarios: infants with birth weights of 3.2 or 3.5 kg, with or without delayed umbilical cord clamping (Table 1; the reference weight at 6 months is based on the WHO Child Growth Standards; World Health Organization Multicentre Growth Reference Study Group, 2006).

At birth and at 6 months of age TBI can be calculated, based on the following equations (Fomon, 1995):

$$\text{TBI} = \text{Fe}_{\text{circ}} + \text{Fe}_{\text{myo/en}} + \text{Fe}_{\text{stores}}$$

where Fe_{circ} is the Fe in circulation as Hb, Fe_{myo/en} is the Fe needed for myoglobin and other Fe-containing enzymes and Fe_{stores} is the amount of storage Fe that is thought to be adequate. Fe_{PT}, the Fe contributed to TBI from placental transfusion, will be an addition to this equation, because although the Fe from placental transfusion is in the form of circulatory Fe, soon after birth it becomes a part of Fe stores. Body Fe at birth is generally estimated to be 75 mg/kg BW, not including the Fe provided with cord clamping (Widdowson & Spray, 1951). However, for purposes of illustration, each body compartment will be calculated when possible.

Total body iron at birth

Fe_{circ0}: assuming a birth weight of either 3.2 or 3.5 kg, a blood volume of 75 ml/kg BW and an average Hb

concentration at birth of 170 g/l (Brown, 1988), the Fe in the circulation can be estimated using the known concentration of Fe, 3.47 mg/g Hb:

$$\begin{aligned} 3.2 \text{ kg infant: } Fe_{\text{circ}0} &= 3.2 \text{ kg BW} \times 75 \frac{\text{ml}}{\text{kg BW}} \\ &\times 0.170 \frac{\text{g Hb}}{\text{ml}} \\ &\times 3.47 \frac{\text{mg Fe}}{\text{g Hb}} = 142 \text{ mg Fe,} \end{aligned}$$

$$\begin{aligned} 3.5 \text{ kg infant: } Fe_{\text{circ}0} &= 3.5 \text{ kg BW} \times 75 \frac{\text{ml}}{\text{kg BW}} \\ &\times 0.170 \frac{\text{g Hb}}{\text{ml}} \\ &\times 3.47 \frac{\text{mg Fe}}{\text{g Hb}} = 155 \text{ mg Fe.} \end{aligned}$$

$Fe_{\text{myo/en}0}$: estimates of body Fe found in myoglobin and enzymes are not as straightforwardly calculated, and estimates from adults are often used. In adult males it is estimated that myoglobin constitutes 10% TBI and Fe-containing enzymes constitute 3% TBI, which together amounts to 13% TBI, which is equivalent to approximately 20% Hb-Fe (Dallman, 1990). Assuming the same relationship holds in infants, the estimate would be 28 mg for the 3.2 kg infant and 31 mg for the 3.5 kg infant.

$Fe_{\text{stores}0}$: at birth Fe stores are large (assuming adequate prenatal nutrition), estimated as 25% TBI (Dallman *et al.* 1993), which corresponds to Fe_{stores} at birth for a 3.2 kg infant of approximately 57 mg and for a 3.5 kg infant approximately 62 mg.

Fe_{PT} : if it is assumed that with delayed clamping an infant receives approximately 40 ml blood/kg BW, the quantity of 'extra' Fe that a late-clamped infant will receive can be estimated. Assuming a Hb concentration of 170 g/l at birth and 3.47 mg Fe/g Hb, delayed clamping would transfer 128 and 140 ml blood and 75 and 83 mg Fe for a 3.2 and 3.5 kg infant respectively. The earlier calculation of TBI concentration before placental transfusion gives a value of 227 and 246 mg for the 3.2 and 3.5 kg infants respectively. The amount of Fe provided by delayed clamping would therefore represent a 30–33% increase in TBI. Although 'immediate' clamping of the cord would theoretically prevent any substantial Fe transfer to the infant, because of the rapid nature of the placental transfusion (25% of the total transfer in the first 30 s), it is likely that an infant undergoing early clamping will receive some 'additional' Fe. Using an estimate of approximately 10% of the total transfer, an infant undergoing early clamping would receive approximately 7–8 mg Fe. Thus, at birth the infant's TBI may range between 234 mg and 331 mg depending on the infant's birth weight and the time of cord clamping.

Total body iron at 6 months

$Fe_{\text{circ}6}$: assuming a weight increase of approximately 4 kg by 6 months of age, and that the infant's blood volume is

75 ml/kg BW (Linderkamp & Versmold, 1977) and a desirable Hb at this age is 118 g/l (Domellöf *et al.* 2001), the Fe in the circulation would be calculated as follows:

$$\begin{aligned} 7.6 \text{ kg infant: } Fe_{\text{circ}6} &= 7.6 \text{ kg BW} \times 75 \frac{\text{ml}}{\text{kg BW}} \\ &\times 0.118 \frac{\text{g Hb}}{\text{ml}} \\ &\times 3.47 \frac{\text{mg Fe}}{\text{g Hb}} = 233 \text{ mg Fe.} \end{aligned}$$

$Fe_{\text{myo/en}6}$: assuming 20% Hb-Fe (Dallman, 1990), Fe in myoglobin and enzymes would be 47 mg for the 7.6 kg infant.

$Fe_{\text{stores}6}$: at 6 months an estimate used for an adequate quantity of Fe in stores is the Fe store concentration found in a normal adult woman of child-bearing age, which is 5 mg/kg BW (Dallman, 1974). The Fe store therefore corresponds to approximately 38 mg for a 7.6 kg infant, which is approximately 10% TBI.

In addition, Fe lost normally through turnover of gastrointestinal and skin epithelial cells, estimated to be a daily loss of 0.05% TBI, must be accounted for (Baynes & Stipanuk, 2000). Breast-fed infants are estimated to lose 0.17 mg Fe/d through the gastrointestinal tract (Schulz-Lell *et al.* 1987). Normal dermal Fe losses in adult males are approximately 0.33 mg/d (Jacob *et al.* 1981), and assuming that an infant has approximately one-twenty-fifth of the surface area of adults (Fomon, 1995), daily dermal losses are estimated to be 0.01 mg. Over 6 months dermal and gastrointestinal losses would therefore amount to 33 mg Fe.

Change in total body iron and absorbed iron needed

Between birth and 6 months the infant needs to absorb a quantity of Fe equivalent to the change in TBI plus additional Fe to account for daily dermal and gastrointestinal losses. The absorbed Fe requirement over 6 months would range from 95 to 118 mg (from 0.52 to 0.64 mg/d) for infants undergoing early clamping, and would be between 20 and 50 mg (between 0.11 and 0.27 mg/d) for infants undergoing delayed clamping, depending on birth weight in both cases (Table 1). Breast milk can provide approximately 0.27 mg Fe/d (assuming a concentration of 0.35 mg Fe/l and 780 ml average daily consumption). Estimates of the percentage of breast-milk Fe absorbed range from 12 to 56 (from 0.03 to 0.15 mg/d; Davidsson *et al.* 1994; Abrams *et al.* 1997; Domellöf *et al.* 2002b). Using the high (56%) and low (12%) estimates of Fe absorption from breast milk the 'net' Fe need from birth to 6 months ranges from zero for an infant undergoing delayed clamping who weighed 3.5 kg at birth to 0.61 mg Fe/d for an infant undergoing early clamping who weighed 3.2 kg at birth. Over 6 months the net need for Fe ranges from 0 to 44 mg for infants undergoing delayed clamping, and from 68 to 112 mg for infants undergoing early clamping.

The calculation of TBI at 6 months presented in Table 1 assumes that some Fe is still in stores at 6 months (approximately 10% TBI), which may not necessarily be the case. It is useful to determine at what age the TBI at

Table 2. 'Reference' iron needed to maintain 'desirable' tissue and circulatory iron during infancy, assuming no iron in stores at 3, 4, 6 and 12 months of age*

	Age (months)				
	2	3	4	6	12
Desirable Fe in tissue and circulation (mg)	199.0	224.8	246.9	280.1	348.5¶
+ Fe losses† (GI and dermal; mg)	10.9	5.5	5.5	10.9	32.8
– Breast milk Fe provided during time period (mg)‡	5.6	2.8	2.8	5.6	16.8
Fe needed to maintain desirable tissue and circulating Fe, accounting for losses and breast milk Fe (mg)	204.3	227.5	249.6	285.4	364.6
Increase in tissue and circulatory Fe (mg; from previous age)		23.1	22.1	35.8	79.2
Net Fe requirement for time period§ (mg/d)		0.76	0.73	0.59	0.43

GI, gastrointestinal.

*For all ages (months), the average of the median weight-for-age (kg) for girls and boys from the WHO Child Growth Standards (World Health Organization Multicentre Growth Reference Study Group, 2006) was used as the reference weight: 2, 5.4; 3, 6.1; 4, 6.7; 6, 7.6; 12, 9.3.

†Fe losses are estimated for the time period previous to the age category (i.e. losses at 2 months are calculated as the sum of losses from birth to 2 months of age, losses at 4 months are calculated as the sum of losses from age 3 months to 4 months). GI and dermal losses are estimated as 0.18 mg/d.

‡Amount of Fe provided by breast milk calculated as the mean of the range of bioavailability (12–56%) amounting to 0.092 mg Fe/d. 'Time period' refers to the interval previous to that age (i.e. 'at 3 months' refers to the period from birth to 3 months).

§Time period refers to the interval previous to that age (i.e. the net Fe requirement at 4 months is for the interval between 3 months and 4 months).

||Assuming blood volume of 75 ml/kg and a Hb concentration of 118 g/l and that myoglobin and enzyme Fe is 20% Hb-Fe.

¶Assuming blood volume of 75 ml/kg and a Hb concentration of 120 g/l and that myoglobin and enzyme Fe is 20% Hb-Fe.

Table 3. Length of time the birth total body iron would be sufficient to maintain desirable levels of tissue and circulatory iron, with no iron in storage, by birth weight and clamping time

Birth wt (kg)	Clamping time		Age (months)					
			Birth†	2	3	4	6	12
		'Reference' Fe needed to maintain desirable tissue and circulating Fe, accounting for losses and breast-milk Fe (mg)	204.3	227.5	249.6	285.4	364.6	
3.2	Early	Body Fe available to meet desirable levels of tissue/circ Fe (mg)*	233.5	228.6	226.0	223.3	217.9	201.9
		Time birth body Fe sufficient (months)			→3.0			
3.2	Delayed	Body Fe available to meet desirable levels of tissue/circ Fe (mg)*	301.5	296.1	293.5	290.8	285.4	269.4
		Time birth body Fe sufficient (months)					→6.0	
3.5	Early	Body Fe available to meet desirable levels of tissue/circ Fe (mg)*	255.8	250.4	247.8	245.1	239.7	223.7
		Time birth body Fe sufficient (months)				→3.9		
3.5	Delayed	Body Fe available to meet desirable levels of tissue/circ Fe (mg)*	330.8	325.4	322.8	320.1	314.7	298.7
		Time birth body Fe sufficient (months)						→8.2

tissue/circ, tissue and circulatory.

*Accounting for Fe losses and the intake of breast milk Fe (see Table 2) at each time point.

†Birth total body Fe calculated as presented in Table 1 for each hypothetical infant.

birth, plus Fe intake from breast milk, would no longer be sufficient to maintain adequate tissue Fe (i.e. stores would be exhausted). Table 2 shows the calculations to determine a 'reference' amount of tissue and circulatory Fe needed at 2, 3, 4, 6 and 12 months of age, accounting for Fe losses and Fe provided by breast milk (the reference weights used in the calculations are based on the WHO Child Growth Standards; World Health Organization Multicentre Growth Reference Study Group, 2006). These reference desirable amounts, and their associated requirements, are then used to determine at what age the previously-examined hypothetical infants would no longer have adequate body Fe to meet these needs (Table 3). For example, the 3.2 kg infant

undergoing early clamping would have approximately 234 mg body Fe at birth. At 2 months approximately 204 mg Fe is needed to maintain Hb and tissue Fe at adequate levels (199 mg needed in tissue and circulatory Fe and 5 mg to account for Fe losses and breast-milk intake, as shown in Table 2), which can be maintained by the birth Fe of this infant (234 mg). However, at 3 months of age 228 mg Fe are needed to maintain adequate tissue and circulatory Fe (225 mg in tissue and circulation and 3 mg to account for Fe losses and breast milk intake, as shown in Table 2). Thus, this same infant would be unable to maintain adequate tissue and circulatory Fe after 3 months of age. In comparison, the infants undergoing delayed

clamping would be able to maintain adequate tissue and circulating Fe for 6.0–8.2 months before entering negative Fe balance. It should be noted that the earlier calculations are based on several assumptions extrapolated from adults (e.g. the amount of Fe in myoglobin and tissue, dermal Fe losses), which may not be appropriate for infants. In addition, some calculations are based on a range of values (e.g. absorption of breast-milk Fe) or of an estimated 'desired' value (e.g. desired Hb concentration or body Fe stores), both of which can introduce error into the final determinations of Fe needs and length of time that birth Fe stores will last.

Effects of iron supplementation of breast-fed infants

As Fe deficiency is common during infancy, prophylactic Fe supplementation is sometimes recommended for breast-fed infants. However, until recently the efficacy and safety of this approach had not been evaluated. Thus, a randomized double-blind placebo-controlled Fe-supplementation trial of breast-fed infants was conducted in two sites: San Pedro Sula, Honduras and Umea, Sweden (Domellöf *et al.* 2001). Subjects were stratified by study site and gender, and randomized to three intervention groups: (1) Fe supplement from age 4 months to 9 months; (2) placebo from 4 months to 6 months and Fe from 6 months to 9 months; (3) placebo from 4 months to 9 months. The Fe supplement was FeSO₄, at a dose of 1 mg elemental Fe/kg per d, adjusted monthly according to infant weight. Term infants with a birth weight >2500 g whose mothers were >16 years of age and intended to exclusively breast-feed for at least 6 months and to continue breast-feeding for at least 9 months were eligible. Infants with Hb <90 g/l or chronic illness were excluded.

In total 131 infants in Honduras and 101 infants in Sweden were followed for ≥ 6 months of age. The characteristics of subjects at the two sites differed; compared with the mothers in Sweden, the mothers in Honduras were younger, had more children and had lower weight and height, and their infants had a lower weight at birth and at 4 months. At 4 months infants in Honduras had lower Hb, mean corpuscular volume and ferritin concentrations and higher zinc protoporphyrin (ZPP; indicative of Fe deficiency) than infants in Sweden. At both sites infants receiving Fe supplements from age 4 months to 6 months were found to have increased Hb and ferritin (and lower ZPP) at 6 months compared with those receiving placebo, regardless of their initial Fe status. Fe supplementation from age 6 months to 9 months was found to increase ferritin and mean corpuscular volume and reduce ZPP at 9 months at both sites, but an effect on Hb was found only in Honduras. The effect of Fe supplementation from 6 months to 9 months on Hb at 9 months was found to be modulated by the Fe status of the infant at 6 months, whereas no such modulation was observed at 4–6 months. This finding suggests that the infant's Hb response to Fe supplementation is immature before 6 months of age, a finding that has been confirmed in rat studies (Leong, 2003; Leong *et al.* 2003).

In Honduras the prevalence of IDA (defined as Hb <110 g/l and two of three Fe status indices (ferritin, mean corpuscular volume, ZPP) abnormal, with cut-off values of <12 µg/l, <70 fl and >80 µmol/mol haem respectively) at 9 months was found to be 29% in the placebo group and 9% in the two Fe-supplemented groups ($P=0.006$), whereas in Sweden no significant effect of Fe supplementation on the already low prevalence of IDA (<3%) was observed. These results indicate that Fe supplementation can effectively reduce IDA in Honduras, where infants are at high risk of Fe deficiency as a result of lower birth weight, maternal prenatal Fe deficiency, hospital policies for early umbilical cord clamping and little use of Fe-fortified complementary foods after 6 months. By contrast, Fe supplementation has no effect on IDA in Sweden, where infants are at lower risk for IDA as a result of higher birth weight, better prenatal Fe status, hospital policies for delayed umbilical cord clamping and extensive use of Fe-fortified complementary foods after 6 months.

The effect of Fe supplementation on growth and morbidity in these infants has also been evaluated (Dewey *et al.* 2002). Among the Swedish infants gains in length and head circumference were found to be lower in those who received Fe than in those given placebo from 4 months to 9 months. The same effect on length was observed in Honduras, but only at 4–6 months among those infants with an initial Hb level of >110 g/l. The main effect of Fe supplementation on morbidity was not found to be significant, but an interaction between supplementation and initial Hb was reported. Among infants with a Hb level of <110 g/l at 4 months (both sites combined), diarrhoea was found to be less common among those given Fe than in those given placebo from 4 months to 9 months, whereas the opposite was true among those infants with a Hb level of ≥ 110 g/l. These results indicate that routine Fe supplementation of breast-fed infants may benefit those with low Hb but poses risks for those who are Fe-replete. Thus, a cautious approach is warranted in relation to routine Fe supplementation during infancy.

Gender differences in iron status during infancy

In the Honduras–Sweden study described earlier substantial differences in Fe status were observed between males and females (Domellöf *et al.* 2002c). At 4, 6 and 9 months of age boys were found to have lower Hb, mean corpuscular volume and ferritin concentrations and higher ZPP and transferrin receptor concentrations than girls. At 9 months the prevalence of IDA (both sites combined) was 17% in boys v. 2% in girls ($P<0.001$). The differences remained significant when controlling for birth weight and rate of weight gain postnatally. The results indicate that the differences in mean corpuscular volume and ZPP may reflect physiological differences in the normal levels of these variables between the genders, but the differences in Hb and transferrin receptor seem to reflect increased true Fe deficiency in boys. Other studies have confirmed that gender may play a role in predisposition to Fe deficiency (Wharf *et al.* 1997; Sherriff *et al.* 1999; Thorsdottir *et al.* 2003; Miller *et al.* 2006). The mechanisms

responsible for this difference are not yet known, but may involve gender differences in the expression of testosterone and oestrogen, which can both affect erythropoietin production, or physiological characteristics that differ between males and females such as lean body mass accretion during infancy, or size of Fe stores at birth.

Diagnostic criteria for iron deficiency and anaemia

The current reference values for Hb and indicators of Fe status during infancy are generally extrapolated from older age-groups and may not be appropriate for infants. In particular, there is mounting evidence that the Hb cut-off of <110 g/l is too high and results in an overestimation of the prevalence of anaemia. To examine this issue, data from the Honduras–Sweden study were used to evaluate appropriate cut-off values for Hb and indicators of Fe status during infancy (Domellöf *et al.* 2002a). Reference ranges were determined using three different approaches for defining Fe-replete infants. The suggested 2 sd cut-off values for Hb were <105 g/l at 4–6 months and <100 g/l at 9 months. Suggested cut-off values for indicators of Fe status (ferritin, ZPP and transferrin receptor) were also presented (Domellöf *et al.* 2002a).

Conclusions

Several conclusions can be drawn from the results discussed herein. The amount of Fe provided from stores at birth plus intake from breast milk can provide sufficient Fe for ≥ 6 months if the infant is full-term, normal birth weight, the mother had adequate prenatal Fe status and the infant underwent delayed cord clamping. Breast-fed infants may be at risk of Fe deficiency before 6 months if any of these conditions do not apply. Fe metabolism undergoes developmental changes during the first year of life. Before 6 months of age the Hb response to Fe supplementation is not regulated, i.e. it occurs regardless of initial Fe status. By 9 months of age regulation is more mature and the response to Fe supplementation is greater in those who are initially Fe deficient. Fe absorption from human milk can vary widely depending on age and Fe status. The range is approximately 12–56%, with absorption increasing as ferritin concentration decreases. After 6 months of age breast-fed infants are at risk of Fe deficiency unless Fe-rich complementary foods are given. Fe supplementation can be beneficial for infants at high risk of IDA, but poses risks for Fe-replete infants, including slower linear growth and increased diarrhoeal morbidity. The appropriate Hb cut-off for anaemia during infancy is <110 g/l; the suggested cut-off is <105 g/l at 4–6 months and <100 g/l at 9 months. Separate cut-off values for male and female infants may be needed for some indicators of Fe status. More research is needed to determine if this gender separation is warranted.

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